THE DIFFERING IMPACT OF CHLOROQUINE AND PYRIMETHAMINE/SULFADOXINE UPON THE INFECTIVITY OF MALARIA SPECIES TO THE MOSQUITO VECTOR

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Abstract. Using serum or infected blood from Danish volunteers and Plasmodium falciparum–infected Mozambican patients, respectively, the impact of curative doses of chloroquine and pyrimethamine/sulfadoxine upon infectivity of P. falciparum to Anopheles arabiensis and An. gambiae or of P. berghei to An. stephensi was studied. Both treatments cleared circulating P. falciparum gametocytes within 28 days. Before this clearance, chloroquine enhanced infectivity to An. arabiensis, whereas pyrimethamine/sulfadoxine decreased infectivity. Patients harboring chloroquine-resistant parasites as opposed to -sensitive ones were 4.4 times more likely to have gametocytes following treatment. In contrast, pyrimethamine/sulfadoxine-resistant parasites were 1.9 times less likely to produce gametocytes. In laboratory infections using replicated P. berghei or P. falciparum preparations, serum from chloroquine-treated, uninfected, nonimmune volunteers enhanced gametocyte infectivity with increasing efficiency for 21 days following treatment, whereas pyrimethamine/sulfadoxine significantly suppressed infectivity. The observed enhancement of infectivity induced by the use of chloroquine combined with increased gametocytamias in chloroquine-resistant strains may in part explain the rapid spread of chloroquine resistance in endemic populations.

Chloroquine-resistant Plasmodium falciparum is now widespread, and reports of resistance by P. vivax to this drug are increasingly more frequent.\textsuperscript{1-3} Mechanisms that may underlie the rapid spread of this resistance are not fully understood. It has been suggested that the asexual blood stages of chloroquine-resistant parasites replicate faster than their sensitive counterparts,\textsuperscript{4} and observations on malaria transmission in Tanzania have been interpreted as suggesting that the introduction of chloroquine is associated with increased transmission to the mosquito vector.\textsuperscript{5} Malaria is transmitted to the mosquito vector by the gametocytes. Mature gametocytes of P. falciparum (stages IV and V) are not sensitive to many schizontocidal drugs, including chloroquine and pyrimethamine/sulfadoxine,\textsuperscript{6} with the result that following treatment of a patient bearing drug-sensitive parasites, mature and infectious gametocytes will persist until the end of their natural life some 14–28 days later.\textsuperscript{7-9} Surprisingly chloroquine, quinine, and some antifolates have been described as increasing gametocytemia and/or infectivity of gametocyte carriers to the vector,\textsuperscript{10-13} although others describe no such influence.\textsuperscript{14-17}

Mechanisms by which drugs might increase the infectivity of gametocyte carriers are not understood but could function by either increasing the number of mature gametocytes circulating in the bloodstream or by increasing the infectivity of individual gametocytes by direct or indirect means.

Previous studies have suggested that chloroquine induces increased gametocytemia in both chloroquine-sensitive,\textsuperscript{18} and more notably, chloroquine-resistant lines.\textsuperscript{19, 20} This has been linked to observed increases in the infectiousness of the gametocyte carrier to the mosquito vector.\textsuperscript{19-21} An immediate chloroquine-induced increase in gametocytemia is allegedly caused by the release of sequestered, mature, drug-insensitive gametocytes;\textsuperscript{22} however, the majority of the observations\textsuperscript{6, 9, 9} are not consistent with this interpretation. Bishop\textsuperscript{22} has similarly argued that sulfadiazine-resistant lines of P. gallinaceum have a higher intrinsic capacity to produce gametocytes.

Increased infectivity in the absence of an increase in gametocytemia has been reported in a chloroquine-resistant (but not a chloroquine-sensitive) strain of P. berghei following treatment of infected mice,\textsuperscript{23-25} although a subsequent study did not wholly support the initial finding.\textsuperscript{26} Such increases could result from the removal of the asexual parasites from the bloodstream, which in turn both increases the hematocrit and reduces the production of parasite-induced serum components that block mosquito infection.\textsuperscript{27} Alternatively, recognizing that direct addition of chloroquine to cultured P. falciparum gametocytes does not enhance infectivity,\textsuperscript{17} whereas addition of drug to the host does,\textsuperscript{11} chloroquine could interact directly or indirectly with the vertebrate host and promote the infectivity of the gametocytes by an as yet undescribed mechanism.

Here we examine the question as to whether chloroquine or pyrimethamine/sulfadoxine increases the intrinsic infectivity of gametocytes persisting in the host following administration of an effective schizontocidal treatment. We used both P. falciparum and the rodent parasite P. berghei. The impact of serum taken from nonimmune Danish individuals who had taken a course of chloroquine or pyrimethamine/ sulfadoxine treatment upon gametocyte infectivity was examined to assess the separate impact of the drugs, their metabolites, or induced host-products upon gametocyte infectivity. These results were compared with observations on Mozambican P. falciparum gametocyte carriers after similar courses of chemotherapy.

MATERIALS AND METHODS

Drug kinetics in control sera. Two healthy adult Danish volunteers took a course of chloroquine (25 mg/kg over a three-day period) and two others took pyrimethamine/sulfadoxine (75 mg of pyrimethamine and 1,500 mg of sulfadox-
ine in a single dose). On day 0 before drug treatment and subsequently on days 4, 7, 14, 21, and 28, blood was collected into Vacutainers without anticoagulant (Becton Dickinson, Glostrup, Denmark). Following centrifugation, serum samples were stored at −80°C for drug analysis and membrane feeding experiments. The sera were analyzed for chloroquine and desethyl-chloroquine28 or for sulfadoxine and pyrimethamine.

Plasmodium berghei infectivity. A chloroquine/pyrimethamine-sensitive P. berghei (clone 2.34) was maintained in outbred Theiler’s Original mice by blood and mosquito passage as previously described.30 Mice were infected with 10⁸ parasites, and 3–4 days after infection, when parasitemia was less than 10%, blood was collected in heparinized syringes (30 units of preservative-free heparin/ml of blood).

The blood was kept on ice and mixed with equal volumes of serum from the drug-treated patients or normal human serum. Parallel aliquots (800 μl) from all samples from a single patient were presented at 37°C to fasting female An. stephensi mosquitoes in membrane feeders. The mosquitoes were then allowed to feed for 45 min, and the unfed mosquitoes were removed. The fed mosquitoes were maintained at 19°C on a fructose/p-amino benzoic acid diet, and given an additional blood feed on uninfected mice on day 4. Ten days after feeding, the infectivity of gametocytes was measured by counting oocysts on mosquito midguts. Infectivity was expressed as the ratio of the arithmetic mean oocyst intensity (%T₀) to the pretreatment sample (T₀).

Plasmodium berghei exflagellation. Two microliters of heparinized P. berghei-infected blood were mixed with equal volumes of the test sera on a microscope slide and covered with a vaseline-edged cover slip. Exflagellation centers were repeatedly counted in 5 × 10⁴ red blood cells per test every 2–4 min between 4 and 30 min after samples were made, and average counts were compared.

Plasmodium falciparum infectivity of cultured gametocytes. Gametocytes of the P. falciparum NF54 strain were cultured in vitro, and on day 14 gametocyte-infected cells were processed for membrane feeding of An. gambiae.31 The washed cells were mixed with control serum (AB+ nonimmune pooled Danish sera) or test serum (nonimmune sera from chloroquine- or pyrimethamine/sulfadoxine-treated healthy Danish individuals, see above).

Plasmodium falciparum gametocyte carriers. The following two aspects of the study, conducted in Matola, Mozambique, were approved by the Ethics Committee of the National Institute of Health (Maputo, Mozambique) and the Ethics Committee of Frederiksberg (Copenhagen, Denmark). Informed consent was obtained individually from the participants or their parents. From August 1995 to October 1996, 36 individuals of all age groups who were identified as P. falciparum gametocyte carriers at the clinic in Matola, Mozambique32 were randomized into two groups. Under supervision, one group (n = 18) was treated with chloroquine, 25 mg/kg over a three-day period, while the other (n = 18) was given pyrimethamine/sulfadoxine tablets (25 mg of pyrimethamine/500 mg of sulfadoxine) as a single dose according to body weight (15–19.9 kg, 1 tablet; 20–29.9 kg, 1.5 tablets; 30–39.9 kg, two tablets; 40–49.9 kg, 2.5 tablets, > 50 kg, three tablets). The patients were monitored clinically and blood was collected on day 0 before treatment and on days 4, 7, 14, 21, and 28 following treatment and examined for parasitemia and hematologic parameters including hemoglobin, white blood cell counts, and platelets. After examination, 5 ml of venous blood was collected into heparinized tubes, centrifuged immediately at 37°C at 500 × g for 5 min, and the plasma and cells were separated. The parasitized cells were reconstituted to a 33% hematocrit with either the patients’ own plasma or the control serum (AB+ nonimmune pooled Danish sera). The blood was then offered to 30–40, 3–5-day-old, colony-reared, local An. arabiensis mosquitoes. The mosquitoes were allowed to feed for about 15 min according to the standard membrane feeding protocol using minifeeders.31 The fed mosquitoes were maintained on a 10% glucose/p-amino benzoic acid supplement solution and kept at 26–27°C and a relative humidity of 76–80%. Eight days after feeding, the fully fed mosquitoes were dissected and their midguts were examined for oocysts. Results are presented as above.

Plasmodium falciparum drug resistance and gametocyte prevalence. From February 1994 to August 1995, 609 individuals examined at the Matola clinic were P. falciparum parasite positive. Those with a body temperature ≥ 37.5°C or a history of fever, a P. falciparum asexual parasite density greater than 1,000/μl, and a negative result in the modified Saker-Solomon test33 for presence of chloroquine or its metabolites in the urine (183 positive) were recruited into the study (n = 234) and assigned randomly into two groups to receive treatment with either chloroquine or pyrimethamine/sulfadoxine. Excluded were individuals with complicated malaria or evident signs of other illness or requiring hospitalization. One group (n = 118) received chloroquine, while the other (n = 116) was given pyrimethamine/sulfadoxine (25 mg of pyrimethamine/500 mg of sulfadoxine) tablets in a dosage given as above. Eighty-three individuals in the chloroquine group and 94 in the pyrimethamine/sulfadoxine group took the prescribed tablets under supervision. Thirty-three individuals were lost to follow-up. A complete follow-up for P. falciparum gametocyte prevalence was available for 128 individuals, of whom 48 were in the chloroquine-treated group, monitored on days 0, 2, 7, and 14, and of whom 68 were in the pyrimethamine/sulfadoxine-treated group, monitored on days 0, 4, 7, and 14. The days of follow-up differed (day 2 versus day 4) only because of the expected slower P. falciparum parasite clearance with pyrimethamine/sulfadoxine.

Statistical analysis. The effect of treatment on the infectivity of P. berghei and P. falciparum was investigated by analysis of variance (ANOVA);34 infectivity was regressed from day 4 and beyond taking into account the drug administered and replicate number.

A logistic regression model35 was used to investigate the prevalence of gametocytes. The following candidate explanatory variables for the probability of having gametocytes at any given examination were included: age, resistance, treatment, days since treatment, and asexual parasitemia on day 0. The results of the analysis are presented as estimated odds ratios based on the model.

RESULTS

Drug kinetics in control sera. Chloroquine and desethyl-chloroquine concentrations peaked on day 4 (0.75 μmol/L...
and 0.35 μmol/L, respectively), and the half-life of chloroquine per se was consistent with previous data. Pyrimethamine, sulfadoxine, and acetylsulfadoxine concentrations peaked on day 4 (490–565 nmol/L, 250–300 μmol/L, and 7.8–9.5 μmol/L, respectively) (Tables 1 and 2).

**Plasmodium berghei infectivity.** The mean relative infectivity of *P. berghei* gametocytes given to mosquitoes in the presence of sera from individuals who had taken chloroquine was never below the T₀ value, and increased progressively and significantly to a peak of 498% on day 21 compared with the pretreatment serum (Table 1). The prevalence of infection (percentage of mosquitoes infected) decreased from 88% on day 0 to 77% on day 4, but then increased to 90% on day 7 and 93% on days 21 and 28. In marked contrast to the effect exerted by serum from treated patients, the direct addition of chloroquine to the infected blood in the membrane feeder at the same concentrations as that found in the patient serum had no impact on the infectivity of gametocytes to *An. stephensi*. When using *P. berghei*-infected blood given to mosquitoes in the presence of sera from persons who had taken pyrimethamine/sulfadoxine, infectivity was almost totally eliminated on day 4 (1%) compared with the pretreatment serum day 0 (Table 2), but it then increased progressively to return to control levels by day 21, at which time pyrimethamine was no longer detectable in the serum. Statistical analysis of the above data by ANOVA showed a significant effect of both time and drug. The infectivity was significantly higher in the chloroquine than in the pyrimethamine/sulfadoxine group (*P* = 0.016), and the infectivity in the former increased significantly with time (*P* = 0.04); in the pyrimethamine/sulfadoxine group, the infectivity was far below the control values on days 4–14, and the infectivity increased above the control value only on day 28.

**Plasmodium berghei exflagellation.** In marked contrast to the pattern of mosquito infection in the serum of chloroquine-treated persons, *P. berghei* exflagellation in vitro was no less on day 4 than on day 0; it decreased in sera from days 7 to 14, then increased again to control values in sera from days 21 to 28.

**Plasmodium falciparum infectivity of cultured gametocytes.** The results of the transmission experiments using sera collected from uninfected volunteers prior to or following administration of chloroquine or pyrimethamine/sulfadoxine are shown in Tables 1 and 2. The overall patterns of infectivity to mosquitoes observed with chloroquine-treated sera reflect those seen in the *P. berghei* studies, with enhancement reaching 400% on day 21. In contrast, with the observations on *P. berghei*, the sera from the pyrimethamine/sulfadoxine-treated volunteers had no detectable effect on the infectivity of cultured *P. falciparum* gametocytes at any time during the course of the study; the results obtained, although showing the same trends as those from the *P. berghei* experiments, were never statistically significant. Whether the differences observed between parasite species relate to the relatively small sample size in the experiments on *P. falciparum* or to the parasite/mosquito combination used has not been determined.

**Field studies on *P. falciparum* gametocyte carriers.** Following chloroquine or pyrimethamine/sulfadoxine treatment of *P. falciparum* gametocyte carriers in Mozambique, the kinetics of serum drug levels were indistinguishable from those of the Danish volunteers (compare Tables 1 and 2 with 3 and 4). Following treatment with either drug, the density of mature sexual stages remained relatively constant until day 4, and then decreased progressively (half-life = 2.4 days) to zero by day 28 (Tables 3 and 4), suggesting that immature forms are fully sensitive to both drugs. Gametocytes from these patients were resuspended in the patients’ own plasma or in control serum and presented to mosquitoes in membrane feeders. When suspended in control serum (pooled AB+ nonimmune Danish sera), infectivity followed a pattern that generally reflected the decreasing number of gametocytes present in the patients’ blood (Table 3), so that infections always ceased by day 21. However, when conversion of gametocytes to oocysts was calculated, we noted that the efficiency of infection decreased more rapidly than gametocyte numbers in both treatment groups, suggesting that the intrinsic infectivity of the gametocytes decreases with the age of the surviving cells.

The pattern of infectivity when the gametocytes were suspended in serum from chloroquine-treated patients varied from that of the controls. The mean relative infectivity was higher on all days, although the variation in relative infectivity between replicates renders each day’s mean observation of no individual statistical significance. In marked contrast, the infectivity observed when the pyrimethamine/sulfadoxine-treated gametocytes were suspended in the patients...
Effects of chloroquine treatment upon the asexual parasitemia, gametocytemia, and infectivity of the gametocytes of *Plasmodium falciparum*–infected patients from Matola, Mozambique

<table>
<thead>
<tr>
<th>Day of sample after treatment</th>
<th>Chloroquine (mean concentration μmol/L)</th>
<th>Desethyl-chloroquine (mean concentration μmol/L)</th>
<th>Asexual parasites/μl (positive/tested)</th>
<th>Gametocytes/μl (positive/tested)</th>
<th>Mean relative percentage infectivity* (control serum)</th>
<th>Mean relative percentage infectivity* (patient plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2,267 (9/18)</td>
<td>249 (18/18)</td>
<td>100</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>4</td>
<td>0.36</td>
<td>0.23</td>
<td>1,772 (1/18)</td>
<td>258 (15/18)</td>
<td>57 ± 16</td>
<td>244 ± 189</td>
</tr>
<tr>
<td>7</td>
<td>0.32</td>
<td>0.22</td>
<td>1,246 (2/17)</td>
<td>120 (16/17)</td>
<td>13 ± 7</td>
<td>162 ± 199</td>
</tr>
<tr>
<td>14</td>
<td>0.11</td>
<td>0.13</td>
<td>1,012 (3/13)</td>
<td>32 (8/13)</td>
<td>8 ± 5</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>21</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>2,048 (3/13)</td>
<td>166 (7/13)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>28</td>
<td>&lt;0.10</td>
<td>&lt;0.10</td>
<td>172 (2/4)</td>
<td>0 (0/4)</td>
<td>Not done</td>
<td>Not done</td>
</tr>
</tbody>
</table>

* See Table 1 for additional information.

Discussion

Analysis of the effects of antimalarial compounds on the infectivity of gametocytes to mosquitoes is complicated by additional and indirect effects of the drugs on the asexual stages, on the host, and on the vector. In the present work we have distinguished between some of these possibilities. In all experiments examining serum from chloroquine-treated persons, infectivity of identical replicates of gametocytes (from either *P. falciparum* cultures or *P. berghei*) was never reduced significantly below that of the controls, but was often increased many-fold above. This was most noticeable in sera taken (from uninfected individuals) 14–28 days after treatment, when chloroquine and its metabolite desethyl-chloroquine were less than the threshold of detectability by the methods used in this study. This increase in

Table 4

Effects of pyrimethamine/sulfadoxine treatment upon the asexual parasitemia, gametocytemia, and infectivity of the gametocytes of *Plasmodium falciparum*–infected patients from Matola, Mozambique

<table>
<thead>
<tr>
<th>Day of sample after treatment</th>
<th>Pyrimethamine (mean concentration μmol/L)</th>
<th>Sulfadoxine (mean concentration μmol/L)</th>
<th>Asexual parasites/μl (positive/tested)</th>
<th>Gametocytes/μl (positive/tested)</th>
<th>Mean relative percentage infectivity* (± SEM)* (control serum)</th>
<th>Mean relative percentage infectivity* (± SEM)* (patient plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3,209 (11/18)</td>
<td>206 (18/18)</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>4</td>
<td>516</td>
<td>283</td>
<td>41 (1/17)</td>
<td>234 (15/17)</td>
<td>65 ± 41</td>
<td>25 ± 20</td>
</tr>
<tr>
<td>7</td>
<td>331</td>
<td>219</td>
<td>41 (1/16)</td>
<td>199 (15/16)</td>
<td>78 ± 57</td>
<td>13 ± 8</td>
</tr>
<tr>
<td>14</td>
<td>103</td>
<td>124</td>
<td>0 (0/16)</td>
<td>178 (10/16)</td>
<td>3 ± 2</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>21</td>
<td>37</td>
<td>61</td>
<td>0 (0/16)</td>
<td>117 (5/6)</td>
<td>0 ± 0</td>
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</tr>
<tr>
<td>28</td>
<td>&lt;25</td>
<td>20</td>
<td>0 (0/4)</td>
<td>0 (0/4)</td>
<td>Not done</td>
<td>Not done</td>
</tr>
</tbody>
</table>

* See Table 1 for additional information.
not only protects the patient, but also has a substantial blocking effect on the infectivity to the mosquito vector of the persistent gametocyte population. This observation is consistent with the established efficacy of pyrimethamine and pyrimethamine/sulfadoxine in suppressing sporogonic development of *P. berghei* and, with slightly lower efficacy, that of *P. falciparum* and *P. vivax*. While our laboratory results on *P. falciparum* are less consistent than the laboratory studies on *P. falciparum*, the results of the field studies are in agreement, i.e., there was a marked suppression of the infectivity of the drug insensitive gametocytes to the mosquito vector. The somewhat divergent results of our laboratory studies on *P. falciparum* are not due to difference in the techniques for mosquito maintenance (both *An. stephensi* and *An. arabiensis* had nutrients added to the sugar feed, in the former case including p-aminobenzoic acid, a known antagonist of pyrimethamine/sulfadoxine) because the effect was not seen in the unsupplemented group. However, the difference may be caused by the small numbers of mosquitoes dissected or the observation that *P. falciparum* is less sensitive to pyrimethamine/sulfadoxine than *P. berghei*. Earlier studies, summarized by Butcher, have suggested that pyrimethamine and sulfadoxine are effective sporontocidal compounds. The greater efficacy of pyrimethamine/sulfadoxine in the field studies versus the laboratory studies therefore suggests that the gametocytes in the drug-treated patients were, due to their prolonged exposure to the drug, less infectious than those exposed for only a brief period in the laboratory studies. It cannot be decided based on this evidence alone whether the drugs are inactivating the gametocytes directly or whether they slowly accumulate within the parasite to levels that will inhibit subsequent DNA replication in the ookinete and oocyst. Previous studies have indicated that sulfadoxine might increase gametocytogenesis in drug-resistant lines of *P. gallinaceum*. We found a 1.9-fold decrease in pyrimethamine/sulfadoxine-resistant lines of *P. falciparum*, whereas chloroquine-resistant lines showed a 4.4-fold increase.

In recent years, *P. falciparum* malaria has returned to areas where it was previously under control. Resistance of the parasite to chloroquine has contributed to a generally worsening situation. The results presented here may explain in part the observation of Lines and others that in Tanzania malaria has become more infectious to the mosquito vector following the introduction of chloroquine and the emergence of chloroquine resistance. Our study provides further reasons for viewing with considerable concern the use of chloroquine as a single drug, particularly in areas where chloroquine resistance has been described, but also in areas where the parasite is sensitive to the compound. Our data emphasize the need to use chloroquine in combination with an effective gametocytocide (e.g., sodium-β-arterolinate) or as shown here, a sporontocide (e.g., pyrimethamine/sulfadoxine). The artemisinin derivatives offer a promising alternative for treatment and control of transmission. Our study further emphasizes that it is essential to pretest the effect of antimalarials on transmission of the parasite to the mosquito vector, using the combination of sera from drug-treated subjects (taken up to four weeks after drug administration) and in vitro membrane feeding of parasites to mosquitoes, before deciding an appropriate regimen for their use in the field.

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### Table 5

Estimated effects of the significant explanatory variables in the logistic regression model for the risk of having gametocytes after treatment with either chloroquine or pyrimethamine/sulfadoxine of *Plasmodium falciparum*-infected patients from Matola, Mozambique

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group in years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–4</td>
<td>1†</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5–14</td>
<td>0.56</td>
<td>0.32–0.78</td>
<td>0.041</td>
</tr>
<tr>
<td>≤15</td>
<td>0.42</td>
<td>0.23–0.76</td>
<td>0.005</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CQ sensitive</td>
<td>1†</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>P/S sensitive</td>
<td>11.87</td>
<td>4.71–29.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>CQ resistant</td>
<td>4.36</td>
<td>1.61–11.8</td>
<td>0.004</td>
</tr>
<tr>
<td>P/S resistant</td>
<td>6.24</td>
<td>3.01–12.9</td>
<td>0.0005</td>
</tr>
<tr>
<td>Days since treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>6.00</td>
<td>3.22–11.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>14</td>
<td>3.28</td>
<td>1.77–6.09</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

*C I = confidence interval. CQ = chloroquine; P/S = pyrimethamine/sulfadoxine.
† Reference values.

**Note:** Infectivity, which was also seen in *P. falciparum*-infected gametocyte carriers, cannot be explained in the laboratory studies by any increase in gametocyte number or in the efficiency of male gametocytogenesis in vitro. The failure to reproduce this enhancement by the direct addition of chloroquine to control serum in this and earlier studies suggests that either undetected chloroquine metabolites or host molecules induced/suppressed by chloroquine may be responsible for the effect observed. The observations of Ramkaran and Peters suggest that the enhancing factors are produced in mice within 12 hr of chloroquine administration to this host.

We have previously reported that in *P. falciparum* gametocyte carriers, a wide range of hematologic parameters, including hematocrit, platelet, and leukocyte numbers, recover to normal values within seven days of treatment, and it has been shown that a low hematocrit is detrimental to mosquito infection. Nonetheless, the fact that an enhancement of infectivity was sustained in the sera of patients 14–28 days after treatment suggests that either undetected chloroquine metabolites or host molecules induced/suppressed by chloroquine may be responsible for the effect observed. The observations of Ramkaran and Peters suggest that the enhancing factors are produced in mice within 12 hr of chloroquine administration to this host. We have previously reported that in *P. falciparum* gametocyte carriers, a wide range of hematologic parameters, including hematocrit, platelet, and leukocyte numbers, recover to normal values within seven days of treatment, and it has been shown that a low hematocrit is detrimental to mosquito infection. Nonetheless, the fact that an enhancement of infectivity was sustained in the sera of patients 14–28 days after treatment suggests that either undetected chloroquine metabolites or host molecules induced/suppressed by chloroquine may be responsible for the effect observed.
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Reference:


